B1
CONT

CGGGGATCCTCTAGGTTGT 3' (SEQ ID NO:27), 50 nmol of each of the dNTP, 10 µl of Vent DNA polymerase buffer (New England Biolabs), and 2 units of DNA Vent polymerase (New England Biolabs). The DNA was denatured for 5 min at 94°C, subjected to 25 cycles each consisting of a 30 sec denaturing step at 95°C, of a 30 sec hybridisation step at 55°C, and a 45 sec elongation step at 72°C, and then elongation at 72°C was continued for 5 min. The DNA fragments from the reaction mixture were digested for 45 min at 37°C with 20 units of BamHI, then for 1 h at 37°C with 20 units of PstI, and finally purified on a QIAquick column. They were inserted into the plasmid pGEM3Z-*petE* prom (described at section 1.2.) digested tot 1 h at 37°C with BamHI then for 1 h at 37°C with PstI, subjected to 0.8% agarose gel electrophoresis, purified on a QIAquick affinity column, dephosphorylated for 1 h at 37°C in the presence of 12 µl of 10X buffer 3 (New England Biolabs) and 5000 units of calf intestine alkaline phosphatase (CIP, New England Biolabs), and finally purified on a QIAquick affinity column. To carry out ligation, 25 ng of the plasmid treated as described above was contacted with 100 ng of the DNA fragments obtained from the PCR reactions, in the presence of 1.2 µl of T4 10X DNA ligase buffer (New England Biolabs) and 400 units of T4 DNA ligase (New England Biolabs) for 1 night at 18°C. Previously prepared *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to the alkaline lysis method, and analysed by enzymatic digestion and gene amplification using the desoxynucleotides 5' CATGCTGCAGACTAGTATCC 3' (SEQ ID NO:26) selected on the promoter and 5' TTGATTTCACGGGTTGGG 3' (SEQ ID NO:28) selected on the *uidA* sequence. Two resulting plasmids pMRT1116 and pMRT1117 were sequenced. The plasmid pMRT1116 contains the promoter MPr1116 (SEQ. ID03), whereas the plasmid pMRT1117 bears the promoter MPr1117 (SEQ. ID04) which differs from MPr1116 by a duplication, in the 5' region of the chimeric promoter, of the "as-1 like" box and its immediate environment, the length of which is 33 bp, as well as the deletion of 3 bp at position -140, -25 and -24, and the replacement of a cytosine with a thymine at position -54 bp (as illustrated by Figure IV).—

2. On page 26 please replace paragraph 1, lines 6-28, with the following:

B2
The fragment SpeI/DraIII of 70 bp containing the two as-2 elements and the as-1 element was obtained from the plasmid pMRT1111. This latter plasmid, which contains a 58 bp sequence corresponding to a duplication of the as-2 element (Lam et Chua, 1989) and an as-1 element (Lam *et al.*, 1989) originating from the 35S RNA CaMV promoter upstream of the minimal pea plastocyanin promoter modified by the addition of a "G" box, was obtained by lb-PCR in the following manner. The single stranded continuous DNA was generated using the following "directional" desoxynucleotides :

- S1= 5'

TTCCCTTCAAACACATACAAATTTCAGTAGAGAAGAACTCATTACTCTTGAGAAACC
TAGAGGATCCCCG 3' (SEQ ID NO: 34)

- S2 = 5'

CACAAAAACCCAATCCACATCTTTATCATCCATTCTATAAAAAATCACCTTCTGTGT
GTCTCTCTTTCGA 3' (SEQ ID NO:35)

B3
CONF
- S5 = 5'

CTGTGGCACATCTACATTATCTAAATCTAAGCCACGTCGGAGGATAACATATTCTTC
CACACATCTTAGCCA 3' (SEQ ID NO:36)

- S7 = 5'

CATGCTGCAGACTAGTGATTGATGTGATATCAAGATTGATGTGATATCTCCACTGAC
GTAAGGGATGACGCATGCCACT 3' (SEQ ID NO:14)--

3. On page 26 and 27 please replace paragraph 2, lines 29-37 on page 26 and lines 1-8 on page 27, with the following:

B3
-- One hundred picomoles (100 pmol) of the desoxynucleotides S1, S2 and S5 were 5' phosphorylated with 15 units of kinase (Amersham) in the presence of 5 µl 10X kinase buffer (Amersham) and 500 pmol of ATP (Sigma), for 30 min at 37°C. The phosphorylated oligodesoxynucleotides were purified by extraction with a volume of phenol, then a volume of phenol : chloroform : isoamyl alcohol (25:24:1 v/v/v) and finally a volume of chloroform : isoamyl alcohol (24:1 v/v), before being precipitated with 1/10 volume 3M sodium acetate pH 4.8 and 2.5 volumes absolute ethanol at -80°C for 20 min then centrifuged at 16060 g for 30 min. The precipitated oligodesoxynucleotides were washed in 70% ethanol, dried, then resuspended in water at a concentration of 10 pmol/µl. In order to link the "directional" oligodesoxynucleotides, the following "guide" oligodesoxynucleotides were used :

- G1= 5' TGTGTTTGAAGGGAATCGAAAGAGAGACACA 3' (SEQ ID NO:37)

- G2= 5' GATTGGGTTTTTGTGTGGCTAAGATGTGTG 3' (SEQ ID NO:38)

- G4= 5' TGTAGATGTGCCACAGAGTGGCATGCGT 3' (SEQ ID NO:39)--

4. On page 27 please replace paragraph 2, lines 22-33 with the following:

B4
-- Finally, PCR amplification of the single stranded continuous DNA obtained was carried out in a GeneAmp PCR System 9700 thermocycle in the presence of 100 pmol of each of the following oligodesoxynucleotide probes 5' CATGCTGCAGACTAGTGGATT 3' (SEQ ID NO: 29), and 5' CGGGGATCCTCTAGGTTTCT 3' (SEQ ID NO: 30), 50 nmol of each of the dNTP, 10 µl of Vent 10X DNA polymerase buffer (New England Biolabs), and 2 units of Vent DNA polymerase (New England Biolabs). The DNA was denatured for 5 min at 94°C, subjected to 25 cycles each consisting of a 30 sec denaturing step at 95°C, a 30 sec hybridisation step at 56°C, and a 1 min elongation step at 72°C, then further elongation at 72°C for 5 min.--

5. On page 28 and 29 please replace paragraph 3, lines 23-36 on page 28 and line 1 on page 29, with the following:

B5
--The ligation was carried out with 30 ng of dephosphorylated plasmid vector pMRT1116 NheI/DraIII and 50 ng of the 70 bp fragment for 15 h at 18°C in a reaction volume of 20 µl in the

B5
CONT

presence of 2.0 µl T4 10X DNA ligase buffer (New England Biolabs) and 800 units T4 DNA ligase (New England Biolabs). Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to alkaline lysis method, and analysed by enzymatic digestion and gene amplification using the desoxynucleotides 5' -CATGCTGCAGACTAGTATCC 3' (SEQ ID NO:26) selected on the promoter and 5' TTGATTTACAGGGTTGGG 3' (SEQ ID NO:28) selected on the *uidA* sequence. The promoter sequence MPr1 146 (SEQ. ID05) of one of these clones was verified by sequencing.--

6. On page 29 please replace paragraph 3, lines 20-37 on page 29 and lines 1-3 on page 30, with the following:

-- The 54 bp fragment PstI/DraIII containing the 44 bp of the as-2 and as-1 elements of the CaMV 35S was obtained from plasmid pMRT1110. The plasmid pMRT1110, which contains a sequence of 44 bp corresponding to the as-2 element (Lam et Chua, 1989) and the as-1 element (Lam *et al.*, 1989) of the 35S RNA CaMV promoter upstream of the minimal pea plastocyanine promoter modified by the addition of a "G" box, was obtained by 1b-PCR in the following manner. The single stranded continuous DNA was formed using the "guide" desoxynucleotides :

- S1 = 5'

TTCCCTTCAAACACATACAAATTCAGTAGAGAAGAACTCATTACTCTTGAGAAACC
TAGAGGATCCCCG 3' (SEQ ID NO: 34)

- S2 = 5'

CACAAAAACCCAATCCACATCTTTATCATCCATTCTATAAAAAATCACCTTCTGTGT
GTCTCTCTTTCGA 3' (SEQ ID NO: 35)

- S5 = 5'

CTGTGGCACATCTACATTATCTAAATCTAAGCCACGTCGGAGGATAACATATTCTTC
CACACATCTTAGCCA 3' (SEQ ID NO: 36)

- S6 =

5'

CATGCTGCAGACTAGTGGATTGATGTGATATCTCCACTGACGTAAGGGATGACGCAT
GCCACT

3' (SEQ ID NO: 13)—

7. On page 30, please replace paragraph 1, lines 4-20, with the following:

--One hundred picomoles (100 pmol) of the desoxynucleotides S1, S2 and S5 were phosphorylated in 5' by 15 units of kinase (Amersham) in the presence of 5 µl 10X kinase buffer

B7

B7
CONF

(Amersham) and 500 pmol ATP (Sigma), for 30 min at 37°C. The phosphorylated oligodesoxynucleotides were purified by extraction with a volume of phenol, then a volume of phenol : chloroform : isoamyl alcohol (25:24:1 v/v/v) and finally a volume of chloroform : isoamyl alcohol (24:1 v/v), before being precipitated with 1/10 volume 3M sodium acetate pH 4.8 et 2.5 volumes absolute ethanol at -80°C for 20 min then centrifuged at 16060 g for 30 min. The precipitated oligodesoxynucleotides were washed in 70% ethanol, dried, then resuspended in water at a concentration of 10 pmol/μl. In order to link the "directional" oligodesoxynucleotides, the following "guide" oligodesoxynucleotides were used :

- G1= 5' TGTGTTTGAAGGGAATCGAAAGAGAGACACA 3' (SEQ ID NO:37)
- G2= 5' GATTGGGTTTTTGTGTGGCTAAGATGTGTG 3' (SEQ ID NO:38)
- G4= 5' TGTAGATGTGCCACAGAGTGGCATGCGT 3' (SEQ ID NO:39)--

8. On page 30 and 31 please replace paragraph 3, lines 33-37 on page 30 and lines 1-26 on page 31, with the following:

B8

--Finally, PCR amplification of the single stranded DNA obtained was carried out in a thermocycleur GeneAmp PCR System 9700 in the presence of 100 pmol of each of the oligodesoxynucleotide probes 5' CATGCTGCAGACTAGTGGATT 3' (SEQ ID NO:29), and 5' CGGGGATCCTCTAGGTTTCT 3' (SEQ ID NO:30), of 50 nmol of each of the dNTP, of 10 μl of 10X Vent DNA polymerase buffer (New England Biolabs), and 2 units Vent DNA polymerase (New England Biolabs). The DNA was denatured for 5 min at 94°C, subjected to 25 cycles each consisting of a 30 sec denaturing step at 95°C, a 30 sec hybridisation step at 56°C, and of 1 min elongation at 72°C, then further elongation at 72°C for 5 min. The DNA fragments of the reaction mixture were digested for 45 min at 37°C with 20 units of BamHI then for 1 h at 37°C with 20 units of PstI, and finally purified on a QIAquick column. They were inserted in the plasmid pGEM3Z-*petE* prom digested for 1 h at 37°C with BamHI then for 1 h at 37°C with PstI, subjected to 0.8% agarose gel electrophoresis, purified on a QIAquick affinity column, dephosphorylated for 1 h at 37°C in the presence of 12 μl of 10X buffer 3 (New England Biolabs) and 5000 units of calf intestine alkaline phosphatase (CIP, New England Biolabs), and finally purified on a QIAquick affinity column. To carry out the ligation, 25 ng of plasmid treated as described above were contacted with 100 ng of the DNA fragments obtained by PCR, in the presence of 1.2 μl of T4 10X DNA ligase buffer (New England Biolabs) and 400 units T4 DNA ligase (New England Biolabs) for 1 night at 18°C. Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to alkaline lysis method and analysed by enzymatic digestion. The promoter sequence MPr1110 borne by this plasmid pMRT1110 was verified by sequencing.--

9. On page 31 and 32 please replace paragraph 2, lines 27-37 on page 31 and lines 1-3 on page 32, with the following:

B9

-- The 54 bp fragment containing the sequences as-2 and as-1 originating from the CaMV promoter was obtained by digesting 25 μg of the plasmid pMRT1110 for 1 h at 37°C with 80

B9
cont

units of PstI, then the generated ends were blunted by the action of Pfu DNA polymerase (Stratagene, La Jolla, USA) according to the supplier's recommendations. The plasmid thus modified was digested for 1 h at 37°C with 4 units of DraIII, and the 54 bp fragment was isolated by electrophoresis on 3% Nu-Sieve agarose gel (FMC, Rockland, USA) and finally purified on a QIAquick affinity column. The ligation was carried out with 30 ng of vector pMRT1117 prepared as described previously and 50 ng of the 54 bp fragment for 15 h at 18°C in a reaction volume of 20 µl in the presence of 2.0 µl of T4 10X DNA ligase buffer (New England Biolabs) and 800 units T4 DNA ligase (New England Biolabs). Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to the alkaline lysis method, and analysed by enzymatic digestion and gene amplification using the desoxynucleotides 5' CATGCTGCAGACTAGTATCC 3' (SEQ ID NO 26) selected from the promoter and 5' TTGATTTACGGGTTGGG 3' (SEQ ID NO 28) selected from the *uidA* sequence. The promoter sequence MPr1147 (SEQ. ID06) of one of these clones was verified by sequencing.--

10. On page 32 please replace paragraph 2, lines 18-32, with the following:

B10

--In order to achieve this, the plasmid pMRT1147 was digested with 20 units of SpeI for 1 h at 37°C, isolated on 0.8% gel agarose, purified on a QIAquick affinity column and religated for 15 h at 18°C in a reaction volume of 10 µl in the presence of 1 µl of T4 10X DNA ligase buffer (New England Biolabs) and 400 units T4 DNA ligase (New England Biolabs). Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to the alkaline lysis method, and analysed by enzymatic digestion and gene amplification using the desoxynucleotides 5' ATTTAGGTGACACTATAG 3' (SEQ ID NO:31) selected from the plasmid and 5' TTGATTTACGGGTTGGG 3' (SEQ ID NO:28) selected from the *uidA* sequence. The promoter sequence MPr1154 (SEQ. ID07) of one of these clones was verified by sequencing.--

11. On Page 33 please replace paragraph 2, lines 17-35, with the following:

B11

--The plasmid pMRT1111, which contains a 58 bp sequence corresponding to a duplication of the as-2 box (Lam et Chua, 1989) and the as-1 box (Lam *et al.*, 1989) from the 35S RNA CaMV promoter placed upstream of the minimal pea plastocyanine promoter modified by the addition of a "G" box, was obtained by 1b-PCR in the following way. The single stranded DNA was produced with the help of the following directional oligodesoxynucleotides :

- S1 = 5'

TTCCCTTCAAACACATACAAATTTCAGTAGAGAAGAACTCATTACTCTTGAGAAACC
TAGAGGATCCCCG 3' (SEQ ID NO: 34)

- S2 = 5'

B11
Cout
CACAAAAACCCAATCCACATCTTTATCATCCATTCTATAAAAAATCACCTTCTGTGT
GTCTCTCTTTCTGA 3' (SEQ ID NO: 35)

- S5 = 5'

CTGTGGCACATCTACATTATCTAAATCTAAGCCACGTCGGAGGATAACATATTCTTC
CACACATCTTAGCCA 3' (SEQ ID NO: 36)

- S7 = 5'

CATGCTGCAGACTAGTGATTGATGTGATATCAAGATTGATGTGATATCT
CCACTGACGTAAGGGATGACGCATGCCACT 3' (SEQ ID NO: 14)--

12. On page 33 please replace paragraph 4, lines 36-37 on page 33 and lines 1-15 on page 34, with the following:

B12
--One hundred picomoles (100 pmol) of the S1, S2 et S5 oligodesoxynucleotides were 5' phosphorylated with 15 units of kinase (Amersham) in the presence of 5 μ l 10X kinase buffer (Amersham) and 500 pmol ATP (Sigma), for 30 min at 37°C. The phosphorylated desoxynucleotides were purified by extraction with a volume of phenol, then a volume of phenol : chloroform : isoamyl alcohol (25:24:1 v/v/v) and finally a volume of chloroform : isoamyl alcohol (24:1 v/v), before being precipitated by 1/10 volume 3M sodium acetate pH 4.8 and 2.5 volumes absolute ethanol at -80°C for 20 min then centrifuged at 16060 g for 30 min. The precipitated oligodesoxynucleotides were washed in 70% ethanol, dried, then resuspended in water at a concentration of 10 pmol/ml. In order to link the directional oligodesoxynucleotides, the following guide oligodesoxynucleotides were used :

- G1= 5' TGTGTTTGAAGGGAATCGAAAGAGACACA 3' (SEQ ID NO:37)

- G2= 5' GATTGGGTTTTTGTGTGGCTAAGATGTGTG 3' (SEQ ID NO:38)

- G4= 5' TGTAGATGTGCCACAGAGTGGCATGCGT 3' (SEQ ID NO:39)--

13. On page 34 please replace paragraph 2, lines 29-37 on page 34 and line 1 on page 35, with the following:

B13
-- Finally, PCR amplification of the single stranded DNA obtained was carried out in a "GeneAmp PCR System 9700" thermocycle in the presence of 100 pmol of each of the oligodesoxynucleotide probes 5' CATGCTGCAGACTAGTGGATT 3' (SEQ ID NO:29), and 5' CGGGGATCCTCTAGGTTTCT 3' (SEQ ID NO:30), 50 nmol of each dNTP, 10 μ l of Vent 10X DNA polymerase buffer (New England Biolabs), and 2 units Vent DNA polymerase (New England Biolabs). The DNA was denatured for 5 min at 94°C, subjected to 25 cycles each consisting of a 30 sec denaturing step at 95°C, of a 30 sec hybridisation step at 56°C, and of 1 min elongation at 72°C, then further elongation at 72°C for 5 min.—

14. On page 35 and 36, please replace paragraph 3, lines 32-37 on page 35 and lines 1-23 on page 36, with the following:

B14

-- The ligation was carried out with 30 ng of pMRT1116 vector prepared as described above and 50 ng of 70 bp fragment for 15 h at 18°C in a reaction mixture of 20 µl in the presence of 2.0 µl of 10X T4 DNA ligase buffer (New England Biolabs) and 400 units of T4 DNA ligase (New England Biolabs). Previously prepared viable and competent *Escherichia coli* DH5α bacteria, were transformed with a third of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to the alkaline lysis method, and analysed by enzymatic digestion and by gene amplification with the help of the universal SP6 oligodesoxynucleotide (5' TAAATCCACTGTGATATCTTATG 3') (SEQ ID NO:32) located in the 5' region of the promoter and the oligodesoxynucleotide 5' TTGATTTCACGGGTTGGG 3' (SEQ ID NO:28) selected on the *uidA* sequence. This cloning strategy enabled the production of 70 bp fragment inserts into the vector in the 5'>3' orientation (the sequences as-2/as-2/as-1 are cloned in the same orientation as in their native promoter), in 3'>5' antisens orientation (an orientation inverse to that present in the CaMV 35S promoter) and in one or more copies. The following synthetic and chimeric promoters could be obtained Using this strategy : MPr1162 (SEQ. ID19), which corresponds to the insertion of a single 70 bp sequence in normal 5'>3' orientation, MPr1163 (SEQ. ID20), which corresponds to the insertion of two 70 bp sequences in the normal 5'>3' orientation, MPr1164 (SEQ. ID21), which corresponds to the insertion of a single 70 bp sequence in inverse or antisens orientation, and MPr1165 (SEQ. ID22), which corresponds to the insertion of four 70 bp sequences in normal 5'>3' orientation. Each one of these clones was verified by sequencing.--

15. On page 37, please replace paragraph 2, lines 10-31, with the following:

B15

-- The ligation was carried out with 30 ng of vector pMRT1147 prepared as described previously and 50 ng of the 70 bp fragment for 15 h at 18°C in a reaction mixture of 20 µl in the presence of 2.0 µl 10X T4 DNA ligase buffer (New England Biolabs) and 400 units T4 DNA ligase (New England Biolabs). Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed by reacting with a third of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with ampicillin (50 mg/l), was extracted according to the alkaline lysis method, and analyzed by enzymatic digestion and by gene amplification using the universal SP6 oligodesoxynucleotide (5' TAAATCCACTGTGATATCTTATG 3' (SEQ ID NO:32)) located in the 5' region of the promoter and the oligodesoxynucleotide 5' TTGATTTCACGGGTTGGG 3' (SEQ ID NO:28) selected from the *uidA* sequence. The following synthetic promoters could be prepared by the preceding method : MPr1167 (SEQ. ID23), which corresponds to the insertion of three 70 bp sequences in normal 5'>3' orientation, MPr1168 (SEQ. ID24), which corresponds to the insertion of two 70 bp sequences in normal 5'>3' orientation and MPr1169 (SEQ. ID25), which corresponds to the insertion of a single 70 bp sequence in normal 5'>3' orientation. Each of these clones was verified by sequencing.--

16. On page 41 and 42 please replace paragraph 2, lines 32-37 on page 41 and lines 1-9 on page 42, with the following:

B16 -- The ligation was carried out by mixing 100 ng of binary plasmid pGA492 prepared as described above and 50 ng of expression cassette for 1 night at 18°C in a reaction volume of 20 µl in the presence of 2 µl of T4 10X DNA ligase buffer (New England Biolabs) and 400 units of T4 DNA ligase (New England Biolabs). Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with tetracycline (12 mg/l), was extracted according to the alkaline lysis method and analysed by enzymatic digestion as well as by gene amplification using the desoxynucleotides 5' ATATGAGACTCTAATTGGATACCGAGGGG 3' (SEQ ID NO:33) selected from the transfer DNA of the binary plasmid and 5' TTGATTTACGGGTTGGG 3' (SEQ ID NO:28) selected from the expression cassette in the *uidA* sequence. The resulting clone was designated pMRT1152.--

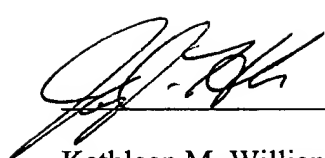
17. On page 43 please replace paragraphs 1 and 2, lines 3-33, with the following:

B17 -- The ligation between the three fragments was carried out by mixing 100 ng of binary plasmid, 50 ng of promoter fragment and 50 ng of the fragment corresponding to the "*uidA*-IV2/*nos* term" sequence in a reaction volume of 20 µl, in the presence of 2 µl of T4 10X DNA ligase buffer (New England Biolabs) and 400 units of T4 DNA ligase (New England Biolabs). The incubation was carried out in a thermocycle by subjecting the ligation mixture to 198 cycles each consisting of a 30 sec incubation at 30°C, and a 30 sec incubation at 10°C. Previously prepared viable and competent *Escherichia coli* DH5α bacteria were transformed with half of the ligation reaction mixture. The plasmid DNA of the obtained clones, selected on LB media supplemented with tetracycline (12 mg/l), was extracted according to the alkaline lysis method and analysed by enzymatic digestion and gene amplification using the desoxynucleotides 5' ATATGAGACTCTAATTGGATACCGAGGGG 3' (SEQ ID NO:33) selected from the transfer DNA of the binary plasmid and 5' TTGATTTACGGGTTGGG 3' (SEQ ID NO:28) selected from the expression cassette in the *uidA* sequence. One of the retained clones was designated pGA492MP1092.

The plasmids pMRT1152, pMRT1171, pMRT1172 and pMRT1182 were transferred into a strain of *Agrobacterium tumefaciens* LBA4404 according to the technique described by Holsters et al. (1978). The plasmid DNA of the obtained clones, selected on LB media supplemented with rifampicin (50 mg/l) and with tetracycline (5 mg/l), was extracted according to the alkaline lysis method, modified by adding lysozyme (25 mg/ml) to the cell resuspension buffer. The plasmid DNA obtained was analysed by enzymatic digestion and gene amplification using the desoxynucleotides 5' ATATGAGACTCTAATTGGATACCGAGGGG 3' (SEQ ID NO:33) selected from the plasmid and 5' TTGATTTACGGGTTGGG 3' (SEQ ID NO:28) selected from the expression cassette. The agrobacteria clones obtained were used to carry out plant genetic transformation.--

May 8, 2002

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